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(54) Title: METHOD FOR TREATING DIABETES			
(57) Abstract The instant invention provides for methods and compositions for the effective treatment of type I diabetes directed towards intervention of interferon alpha activity.			

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Method for Treating Diabetes

Background of the Invention

Field of the Invention

5 This invention is in the field of the prevention and treatment of diabetes mellitus.

Description of the Prior Art

Insulin dependent diabetes (also known as Type I or juvenile diabetes) is a disease characterized by reduced or absent pancreatic secretion of insulin. Type 1 diabetes, is
10 associated with the progressive loss of pancreatic β cells and with several autoimmune disorders (W. Gepts and P.M. LeCompte in The Diabetic Pancreas (B.W. Volk and E.R. Arguilla, eds.), Plenum, New York 1985, pp337-365; G.S. Eisenbach, *New Engl. J. Med.* 314:1360,1986; A.K. Foulis, *J. Pathol.* 152:141,1987). This type of diabetes is sometimes initiated during a pregnancy.

15 Genetic factors, auto-immunity and (possibly) viral infection may also be involved. Stein and Ziff have shown that adenovirus type 5 infection to an insulin producing cell line, HIT T-15 represses insulin II gene transcription in the rat (Stein and Ziff, *Mol. Cell. Biol.* 7:1164, 1987). Certain particular alleles closely linked to the major histocompatibility complex (MHC) class II locus is known to increase the risk of developing type 1 diabetes
20 (J.A. Todd, J.I. Bell, *et al.*, *Nature* 329:599, 1987; G.T. Horn, T.L. Bugawan *et al.*, *Proc. Natl. Acad. Sci USA*, 85:6012, 1988; H.A. Erlich *et al.*, *Diabetes* 39:96, 1990; reviewed in Castano and Eisenbarth, Type-I Diabetes, *Ann. Rev. Immunol.* 8:647-679, 1990).

Several publications describe the possibility that certain virus infections might represent environmental factors that induce the disease (G. Frisk *et al.*, *J. Med. Virol.*
25 17:2919, 1985; M.S. Eberhardt *et al.* *Diabetes Res.* 9:125, 1988; G. Frisk *et al.*, *J. Infect.* 24:13, 1998). However, there is usually no active viral infection directly related to the onset of diabetes type 1.

No satisfactory model has yet been developed which explains how the disease develops. Currently, the only treatment generally available is the exogenous delivery of
30 insulin. Transplantation of islet cells has been attempted, but not all patients are appropriate for this treatment. Further, there is evidence that the transplanted cells only survive for relatively short periods of time, after which the patients revert to exogenous insulin

dependency (Tydén *et al.*, *Transplantation Proceedings* 24: 771, 1986).

Other than in certain lymphocytes, cytokines (e.g., interferon- α) can be induced in certain epithelial cells as well, and among these epithelial cells are pancreatic β cells (A.R. Collins, *Adv. Exp. Med. Biol.* 276:497, 1990). Interferon- α can induce NK cell activity (G. Semenzato *et al.*, *Blood* 68:293, 1986; P. Conti *et al.*, *J. Immunopharmacol.* 10:907, 1988; M. Sarzotti *et al.*, *Nat. Immun. Cell Growth Regul.* 8:66, 1989; A. Verhagen *et al.*, *Nat Immun. Cell Growth Regul.* 9:325, 1990; B.-L. Li *et al.*, *Cancer Res.* 50:5328, 1990; M.T. Kasain and C.A. Biron, *J. Exp. Med.* 171:245, 1990; S.A. Chen *et al.*, *J. Interferon Res.* 8:597, 1988). The same cytokine induction phenomenon can affect the macrophages as well (Y. Tabata *et al.*, *Japan J. Cancer Res.* 79:636, 1988; S. Ellermann-Eriksen *et al.*, *J. Gen. Virol.* 70:2139, 1989; O.N. Ozes *et al.*, *J. Interferon Res.*, 12: 55, 1992). Interferon- α can also modify the antibody response (M. Peters *et al.*, *J. Immunol.* 137:3163, 1986; S.S. Evans and H. Ozer, *J. Immunol.* 138:2451, 1987; F.D. Finkelman, *J. Exp. Med.*, 174:1179, 1991; A. Schnatter *et al.*, *Clin. Immunol. Immunopathol.* 38:327, 1986).

In human diabetics the early lesions of the β cells show infiltration with mononuclear cells, although in the early stages the diabetics may still have insulin producing cells; and a minor infiltration into the surrounding acinar tissue is often found (W. Gepts and P.M. LeCompte, In The Diabetic Pancreas (B.W. Volk and E.R. Arguilla, eds.) Plenum, New York, 1985, pp337-365; G.S. Eisenbarth, *N. Engl. J. Med.* 314:1360, 1986; A.K. Foulis, *J. Pathol.* 152:141, 1987).

Although human diabetics lose almost all their β cells the absolute number of islet cells that stain for glucagon or somatostatin is relatively unchanged. Normally, induction of interferon- α is transient but in diabetic patients who had been clinically diabetic for several months, interferon- α is present in the pancreas (E. DeMayer *et al.*, in Interferons and other Regulatory Cytokines (Wiley, New York 1988) Chapt. 3, pp39-66; A.K. Foulis *et al.*, *Lancet* II:1423, 1987).

With this background in mind, the instant invention teaches methods and compositions for effective treatment of type I diabetes by intervening with the interferon- α activity in diabetics. This type of treatment for diabetes is relevant in human diabetics for several reasons. It is known that β cells of type I diabetes expresses interferon- α rather than interferon beta. Further, it is believed that the islets of human diabetics type I show histologically obvious changes before lymphocytes producing interferon- α , appear (A.K.

Foulis *et al.*, *Lancet* II:1423, 1987). Thus, islet cell damage is induced by endogenously produced interferon- α production, and this production becomes the preferentially expressed transcript overwhelming and inhibiting insulin production.

5 Summary of the Invention

The instant invention provides for a method for treating type I diabetes comprising intervention of interferon alpha activity. Thus, in one embodiment the instant invention encompasses methods for treating early type I diabetes comprising administering an effective inhibitory amount of an antisense oligonucleotide directed to the mRNA encoding for
10 interferon- α . Effective inhibition encompasses the complete or partial inhibition of transcription of the interferon alpha gene, or complete or partial inhibition of translation of the interferon alpha mRNA transcript.

The method of the instant invention also encompasses administering an effective inhibitory amount of anti-interferon- α antibodies. Such antibodies, by binding specifically to
15 interferon- α would be effective in inhibiting the inflammatory effects of the cytokine. Effective inhibition by the specific binding of anti-interferon alpha antibodies can also be accomplished by active binding fragments of such antibodies (such as Fab, Fab², and single chain antibodies) where said antibodies are either monoclonal or polyclonal.

The instant invention also encompasses a method for treating diabetes comprising
20 administering an effective therapeutic amount of anti-interferon- α antibodies in combination with antisense oligonucleotide directed to the mRNA sequence encoding interferon- α .

The instant invention also encompasses intervention in the regulatory pathway which controls interferon production or other related cellular regulatory protein activity (i.e. enzymes), and responses to interferon alpha. In one particular embodiment, the method of the
25 instant invention embodies inhibition of anti-viral cellular response enzyme activity by specific binding. Such targeted anti-viral cellular enzymes include 2',5' oligoadenylate synthetase (2',5' A synthetase), the La antigen (p47,6), and protein kinase p68 (PKR). Inhibitory specific binding may be accomplished by specific monoclonal or polyclonal antibody and binding fragments thereof. In one embodiment the inhibition is accomplished by
30 dsRNA molecules which are competitively and selectively bound to the enzyme molecule. Such inhibitory dsRNA molecules can contain one or more substituted nucleic acids whereby the overall molecule is stabilized to resist degradation.

between interferon- α and a substance (e.g. a monoclonal or polyclonal antibody or an antibody fragment raised against interferon- α) which binds to interferon- α in such a way that the cell inhibiting effects of this cytokine are blocked or inhibited. Such a block or inhibition should of course be specific, *i.e.* the effect must be ascribable to a specific binding between
5 the substance and interferon- α and not merely ascribable to non-specific interference of the substance on a wide variety of binding phenomena.

Another method encompassed by the instant invention is to interfere with the production of interferon- α . This can be accomplished by direct interference with the genetic material encoding interferon- α or by interfering with the transcription products of this genetic
10 material using the antisense technique (Gustafsson *et al.*, *Immunol. Rev.* 141: 59, 1994; all references described are incorporated by reference in their entirety). As antisense fragments both DNA and RNA fragments may be used, but also PNA fragments (peptide nucleic acids; Nielsen P. E. *et al.*, *Science* 254:1497-1500, 1991) are also candidates for use in such therapy, as these molecules have been demonstrated to exhibit superior and very dynamic
15 hybridization profiles. An additional method would be to inactivate the interferon- α gene by a gene knock-out technique described in EP-A-546073 (GenPharm International, Inc; incorporated by reference). A successful inhibition of interferon- α activity may also be accomplished by the effective alteration of the active residues involved in effecting binding.

It is known that interferon expression is one aspect of a generalized pro-inflammatory
20 immune mechanism where both gene expression and induced cell regulatory proteins (*i.e.* enzymes) are involved. Interferon directed expression of certain cell regulatory proteins (*i.e.* enzymes) and the inherent dependency of these proteins on double-stranded RNA molecules (viral or synthetic origin) for induction and regulation of their activity are prime targets for the method of intervention of the instant invention. Most important as targets for intervention,
25 of the genes expressed during the viral defense response, are the 2',5' oligoadenylate synthetase (2',5' A synthetase), the protein kinase p68 (PKR), and the La antigen (p47,6) genes and related transcripts. The dsRNA dependent 2',5' A synthetase exists in five isoforms localized in the cytoplasm (p42, p46), ribosomes (p100), and cell membranes (p69/71). The 2',5' A synthetase link ATP molecules together by 2',5' di-ester bonds
30 yielding 2',5' oligomers of different chain lengths which are specific activators of the RNA degrading latent ribonuclease (RNase L). Thus, the activity of these enzymes is responsible for the breakdown of viral RNA. The genes coding for p42, p46 and p69/71 have been

cloned.

The PKR enzyme is expressed in most mammalian cells and is associated with ribosomes, and also found within the nucleolus. The PKR gene has been recently cloned. Two domains of the protein bind dsRNA with high-affinity leading to auto-phosphorylation
5 whereby the enzyme becomes activated. The activation of PKR by synthetic, viral or cellular dsRNA phosphorylates the eukaryotic protein synthesis initiation factor (eIF-2), which thereby becomes activated. The effect of this process is to produce an anti-viral state via inhibition of cellular protein synthesis and thus inhibition of viral protein synthesis. Over expression of PKR has a significant anti-proliferative effect and may eventually lead to cell
10 death.

RNA molecules have been found to play a significant role in the regulation of PKR (i.e. Epstein-Barr virus, EBER-1 adenovirus VA₁RNA). Viral small RNAs are capable of inhibiting PKR activity by competitive interference with dsRNA binding to the enzyme. The 46.7 kDa cellular La antigen has been shown to have a DNA-RNA and dsRNA unwinding
15 activity resulting in single-stranded RNA which cannot activate PKR. Therefore, viruses with La antigen will escape the degrading effects of the PKR enzymes and replicate uninhibited, and there is increased risk of incorporation of viral genome into host cell genome and/or persistent generation of virus particles in host cell cytoplasm. This may lead to perpetual viral antigen presentation and processed viral neoantigen presentation to immune competent T-
20 lymphocytes.

A transgenic mouse model has been developed to initiate an autoimmune response model for diabetes, capable of destroying β cells when the β cells are made capable of co-expressing interferon- α , thus giving a syndrome comparable to diabetes type 1.

This was done by generating transgenic mice that carry a transgene containing the
25 regulatory region of the human insulin gene, and a cDNA that encodes a hybrid human interferon- α , because ordinary interferon- α is not active in mice (S.A. Chen *et al.*, *J. Interferon Res.* 8:597, 1988; L. Martin *et al.*, *J. Immunol.* 150:1234, 1993; O. Bohoslawec *et al.*, *J. Interferon Res.* 6:207, 1986). Furthermore, the polyadenylate addition signal from hepatitis surface antigen was also transferred. This general form of a transgene can
30 efficiently drive expression of a heterologous gene in the β cells (N. Sarvetnick *et al.*, *Cell* 52:773, 1988).

These mice were called Iia founder mice, which were then backcrossed to outbred

albino mice (CDI) and to inbred mice (C57BL/6). The Iia x CDI transgenic mice became diabetic at a rate of more than 50%, with a median time to onset of the type 1 diabetes of 4 to 6 weeks. The incidence in Iia x C57BL/6 mice was less than 5%. One major consequence of the co-expression of interferon- α transgene in β cells was to induce development of hypoinsulinemic diabetes.

An additional strain of mice called NOD (non-obese diabetic mice) are another model system which can be utilized to demonstrate the effectiveness of the methods of the instant invention. NOD mice are described in detail in Wicker et al., *Ann. Rev. Immunol.* 13:179-200.

Also, spontaneously diabetic BB rats (BioBreeding rats; Petersson et al., *Nature Genetics* 2:56-60) are another model system by which early intervention to prevent the effects of interferon, as a means of preventing the development of diabetes can be tested where there is a demonstrated genetic predisposition to develop diabetes.

The following examples are meant by way of illustration of the methods and compositions of the instant invention and are not intended by way of limitation to be the exclusive embodiment of the instant invention. One of ordinary skill in the art, using the teaching of the instant specification will be able to understand and practice the instant invention and equivalents thereof with only routine experimentation.

EXAMPLE 1

Intervention targeted at interferon alpha

It is not known whether the insulin producing β cells, when induced for interferon- α production, produce one or several species of interferon- α such as the buffy coat leukocytes do when induced. However, it is known that certain cells like human lymphoblastoid cells (Namalwa cells) produce only 8 of the ≥ 15 isoforms of interferon- α human species that are produced by the pancreatic insulin producing β cells. In 37 pancreas removed at necropsy from patients with juvenile diabetes, 34 had residual β cells. In 33 of the 34 individuals the insulin producing β cells were positive for interferon- α (Foulis, A. et al., (1987) *Lancet* II, 1423). The antibody used for the detection of interferon- α in these islets was raised by immunizing sheep with lymphoblast interferon (Hu INF- α Ly Namalwa: Wellferon from Wellcome Research Laboratories, Beckenham, UK) an antigen. The hyper-expression of the major histocompatibility complex class I (MHC Class I) appeared to be related to the presence of interferon- α in the islets. Of islets showing hyper-expression of MHC I, 93%

contained interferon- α , compared to only 0.4% of those islets showing no hyper-expression. Interferon- α is known to enhance MHC class I expression by pancreatic endocrine cells in vitro (Pujol-Borell R. et al., Clin. Exp. Immunol. (1986) 65, 128).

Part of this invention is the identification of the species of interferon- α that are produced by the β cells, because the anti-interferon- α species specific antibody(ies) that preferably should be administered into the patients could be targeted specifically by tailor-making the antibody specificity to the certain species of interferon- α produced. The interferon- α family consists of 24 or more genes or pseudogenes. Two families of interferon- α are distinguishable (type I and II) consisting of ≥ 1 of bovine type I. Mature type I interferons α are 166 amino acids long (one is 165 amino acids long), type II is 172 amino acids long.

More than 24 non-allelic genes or pseudo-genes for human interferon- α have been identified. Eighteen of these including at least 4 pseudo-genes are for IFN α (type I), and 6 of the genes of which at least 5 pseudo-genes are for IFN α (type II).

Amino acid sequence for human interferon α D (IFN α type I) and for human interferon α II (IFN α (II) (IFN ω) is given below and is from Swissprot.

Amino acid sequence for human interferon- α D (IFN α type I) (Mantei N. et al., Gene (1980) 10, 1; Zoon K. Interferon (1987) 9, 1):

Accession code: Swissprot PO1562 (SEQ ID NO:1)

```

-23  MASPFALLMV LVVLSCKSSC SLG
    1  CDLPETHSLD NRRTLMLLAQ MSRISPSSCL MDRHDFGFPO EEFDGNQFQK
   51  APAISVLHEL IQQIFNLFTT KDSSAAWDED LLDKFCTELY QQLNDLEACV
  101  MQEERVGETP LMNADSILAV KKYFRRITLY LTEKKYSPCA WEVVRAEIMR
  151  SLSLSTNLQE RLRRKE

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Conflicting sequence A->V at position 114 (Goeddel D.V. et al., Nature (1981) 290, 20).

Amino acid sequence for human IFN α (type II) also called IFN ω (Capon D.J. et al. Mol. Cell. Biol. (1985), 5, 768):

Accession code: Swissprot P05000 (SEQ ID NO:2)

```

-23  MALLFPLLAA LVMTSYSPVG SLG
5   1   CDLPQNHGLL SRNTLVLLHQ MRRISPFLCL KDRRDFRFPQ EMVKGSQQLQK
    51  AHVMSVLHEM LQQIFSLFHT ERSSAAWNMT LLDQLHTELH QQLQHLETCL
    101 LQVVGEGESA GAISSPALTL RRYFQGIRVY LKEKKYSDCA WEVVRNEIMK
    151 SLFLSTNMQE RLRSKDRDLG SS

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10 Disulphide bonds between Cys 1-99 and 29-139.

Gene Structure (Shaw G.D. et al., Nucl. Acids. Res. (1983) 11, 555; Capon D.J. et al., Mol. Cell. Biol. (1985) 2, 768; Mantei N. et al. Gene (1980) 10, 1).

15 In theory the insulin producing β cells are in a constant activated interferon- α producing state. It is remarkable that this active state is not an on - off mechanism as in other interferon- α producing cells and, furthermore, it is also remarkable that the repressor proteins produced when interferon- α is induced in cells which normally would participate in shutting off the mRNA for interferon within hours, does not elicit the same repressor function on the production of interferon- α product in β cells. There could be at least one or more hypothetical

20 reasons for this phenomenon that the interferon- α mRNA in the β cells is not shut off by repressor proteins. One reason could be that the repressor proteins for some reason does not down regulate in β cells, may be due to differences in penetration in these types of cells versus other interferon- α producing cells; another reason could be that the interferon- α is a type such as for instance type II (bovine type), induced by virus.

25 However, there are exceptions; in some cases a spontaneous IFN production in the absence of any added inducers can occur in cultures of macrophages, of lymphoblastoid cells and of leukemic and normal peripheral leukocytes (Northrop R. and Deinhardt F., J. Nat. Cancer Inst. (1967) 39, 685; Pickering L. et al., Proc. Natl. Acad. Sci. USA (1980) 77, 5938; Ablashi D. et al., Proc. Soc. Exp. Biol. Med. (1982) 171, 114; Pickering L. et al. Arch. Virol.

30 (1983) 75, 201). These cells are all derived from the hemopoietic system, but spontaneous low-level IFN production can also occur in cells of other origins, for example, in continuous lines of mouse L or C-243 cells. It is not known whether this is the result of production of

significant IFN by a few cells in the population or of low production by many cells. Low levels of HuIFN α 1 and IFN α 2 mRNA, but not of several other IFN α subtypes or IFN β , are constitutively transcribed in spleen, kidney, and peripheral blood lymphocytes of normal individuals (Tovey M. et al., Proc. Natl. Acad. Sci. USA (1987)). The same phenomenon
5 could be localized to the insulin producing β cells in pancreas.

Low level IFN production resulting in demonstrable virus resistance can be rendered permissible by treatment of anti-IFN α serum or of IFN α/β serum (Jarvis A. and Colby C., Cell (1978) 14, 355; Gresser I. et al., J. Virol. (1985) 53, 221). Therefore antibodies to IFN α , to subtypes of IFN α , or to IFN α/β serum can interrupt a spontaneous IFN production or a
10 induced IFN production that will not be interrupted even if the inducer(s) have disappeared. Hence, it is conceivable that IFN production in insulin producing β cells can be interrupted by antibodies. It is therefore important to establish what subtype of IFN α (and, may be IFN β) the insulin producing β cells are producing. Growth factors and cytokines have been shown to induce the synthesis of IFN α and/or IFN β such as colony stimulating factor 1 (CSF-1)
15 (Sherr, C. et al., Cell (1985) 41, 665); Moore R. et al., Science (1984) 223, 178). PDGF (platelet-derived growth factor) can induce Mu IFN β mRNA in confluent monolayers of murine BALB/c 3T3 cells (Zullo J. et al., Cell (1985) 43, 793). Interleukin 1 (IL-1) (and tumor necrosis factor (TNF) are regulatory cytokines with pleiotropic activities which in human diploid fibroblasts can induce the synthesis Hu IFN β . Interleukin 2 (IL-2), vital for T-
20 cell function can induce IFN in bone marrow cells, and IFN γ can sometimes act as an inducer of IFN α . Part of the antiviral activity induced in mouse L-929 cells by IFN γ can be neutralized by monoclonal antibody to Mu IFN α (Hughes T. and Byron S., J. Biol. Regul. Homeostat. Agents (1987) 1, 29).

In eukaryotes, mRNA is synthesized as a large precursor molecule in the nucleus.
25 The mature mRNA moves to the cytoplasm for translation. For many eukaryotes mRNA is the functional half-life in the cytoplasm can be relatively long, up to 24 hours. Some mRNAs however, have much shorter half-lives, of 30 minutes or lower. Rapid degradation of mRNA in the absence of further transcription is one way of quickly terminating the synthesis of a protein (Caput D. et al., Proc. Natl. Acad. Sci. USA (1986) 83, 1670; Shaw G. and Kamen R.,
30 Cell (1986) 46, 659).

Superinduction can enhance the translation or the transcription or the combination of both. Stabilization of cytoplasmic IFN and mRNA probably contributes to the enhanced and

prolonged synthesis of Hu IFN β in polyIrC - induced human fibroblasts that have been treated with cycloheximide and Actinomycin D in a superinduction scheme; in cells not exposed to these metabolic inhibitors, active degradation of IFN mRNA starts a few hours after induction after induction by polyIrC, whereas in superinduced cells IFN synthesis goes on for several more hours (Raj N. and Pitha P., Proc. Natl. Acad. Sci. USA (1983) 77, 4918).

Other mechanisms than the formation of dsRNA during interferon synthesis induced by an RNA virus infection is conceivable. One can for example induce HuIFN α in peripheral blood mononuclear cells by exposing them to glutaraldehyde fixed cells expressing at their surface herpes simplex or Newcastle disease virus antigens (Capobianchi M. et al., Proc. Soc. Exp. Biol. Med. (1985) 178, 551). In particular instance an interaction between a virus surface component and the mononuclear cell membrane is sufficient to induce an IFN α synthesis.

The glycoproteins of Sendai virus are capable of inducing acid stable IFN α type I in human leukocytes, human lymphoblastoid cells (Namalwa), in murine spleen cells, but are unable to do so in mouse L fibroblasts (Ito Y. et al., Virology (1978) 88, 128). However, for the time being, no hard facts indicates that IFN induction can or should be explained by a universal inducer which would be a common denominator of the many agents, viral and others, shown to stimulate IFN α/β synthesis.

IFN inducers activate several other genes at the same time. In human fibroblasts, polyIrC induces, in addition to Hu IFN β , the synthesis of at least 13 and maybe as many as 23 other proteins, which have not been further characterized (Raj N. and Pitha P., Proc. Natl. Acad. Sci. USA (1980) 77, 4918; Content J. et al., Proc. Natl. Acad. Sci. USA (1982) 79, 2768). Some of these co-induced genes are probably linked, but unrelated to the IFN gene or chromosome 9, Gross G. et al., In: The Biology of the Interferon System (eds. E. DeMaeyer, G. Gallasso, and H. Schellekens) Elsevier, Amsterdam, 1981, pp. 85-90). Gene expression in eukaryotic cells is controlled at several levels. To allow for transcription, the gene must be in an active state, which requires changes in the chromatin structure to permit access of RNA polymerase. Acquisition of the "active" state is the first requirement for gene activation and has in fact been shown to occur in IFN genes upon induction.

The second step of gene activation consists of initiation of transcription and is controlled by the promoter region of the structural gene. The leftward boundary of the promoter is always upstream of the TATA box, a ubiquitous sequence of 7 or 8 bp that lies

usually about 20 to 30 bp upstream from the starting point of transcription (Wagner R., Nature (1964) 204, 49). Functional IFN mRNA cannot be extracted from uninduced cells. Transcription starts early after induction, the actual time being a function of the inducer-cell system studied. When human lymphoblastoid Namalwa cells are treated with the Sendai virus, IFN β and IFN α are produced (Havell E. et al., J. Gen. Virol. (1977) 38, 51). Detectable levels of mRNA are present 3 hours after the onset of induction, reach a maximum at 9 hours and then decline (Shuttleworth J. et al., Eur. J. Biochem. (1983) 133, 399). The cause of the shutoff of IFN synthesis is unknown; it occurs in cell cultures and in the animal and is usually followed by a period of hypo-responsiveness to renewed induction. This does not seem to be the case with regard to the insulin producing β cells. It is unlikely that a negative feedback by IFN itself is responsible for the shutoff or arrest of the synthesis since with very high doses of IFN increases rather than decreases induction (DeMaeyer-Guignard J. et al., Virology (1980) 102, 222). More likely candidates for the shutoff function are provided by the many proteins whose synthesis is coinduced with IFN by viruses or polyI:C.

EXAMPLE 2

Substances found in crude interferon that can decrease IFN production

Crude IFN preparations often contain one or several substances that can decrease IFN production (Friedman R., J. Immunol. (1966) 96, 872). These substances will be effective inhibitors of IFN α , as used in the present invention. It is possible to isolate and characterize these substances, using known techniques, in order to demonstrate that these substances are able to arrest the synthesis of IFN in insulin producing β cells. In fact, Demethylation of cytosine at CpG sites plays a role in the control of eukaryotic gene expression (Felsenfeld G. and McGhee J., Nature (1982), 296, 602). For example, treatment of Namalwa cells with a demethylating agent such as 5-azacytidine is sufficient by itself to induce IFN production, and furthermore, causes a 50-fold increase of NDV induced IFN α production (Tovey M. et al., In: The Biology of the Interferon System, E. DeMaeyer and H. Schellekens, eds.) Elsevier, Amsterdam, 1983, pp. 45-50). The rate of transcription of the IFN genes can also be influenced by IFN itself, a phenomenon known as "priming" (Stewart II, W. et al., J. Virol. (1971) 7, 792). This loop-back mechanism may also be the reason why insulin producing β cells continue synthesis of IFN. Substances can be found in crude interferon shown to repress interferon production may thus play a role in interrupting this function. Furthermore,

antibodies to IFN may be capable of preventing this loop-back-priming phenomenon. Besides priming of interferon by interferon, because transcription of other genes than IFN genes, clustered near the IFN gene are also affected by priming (Nir U. et al., J. Biol. Chem. 1985), 260, 14242).

5

EXAMPLE 3

Control of IFN Synthesis at the posttranscriptional level

For many eukaryotic mRNAs the functional half-life in the cytoplasm can be relatively long, up to 24 hours. Some mRNAs, however, have much shorter half-lives, of 30 minutes or less. mRNA stability is one of the factors intervening in the control of protein synthesis, and rapid degradation of mRNA in the absence of further transcription is one way of quickly terminating the synthesis of a protein. Normally, this appears to be the case for IFN, since IFN mRNAs in general are rapidly degrading mRNAs. In this respect they are comparable to mRNAs of other cytokines and lymphokines and of some proto-oncogenes. These mRNAs all share an AU - rich region in their 3' noncoding region, and the possibility has been raised that this common structural feature is a recognition signal for rapid mRNA degradation (Caput D. et al., Proc. Natl. Acad. Sci. USA (1986) 83, 1670; Shaw G. and Kamen R., Cell (1986) 46, 659).

The HuIFN α 1 gene promoter region contains the necessary information: when the 675 bp upstream of the IFN α 1 coding region are joined to the transcription unit of the rabbit globin gene, and mouse cells are then transformed by this hybrid gene. If, on the other hand, the globin promoter is fused to the IFN α 1 coding sequence, correct transcription no longer depends on viral induction, but instead, has become constitutive. It has been revealed that the region required for inducible transcription is located between positions -117 to -74. This region contains a purine-rich stretch of 42 bp. located immediately downstream of position -117, which is highly conserved in all HuIFN α genes (Benjamin R. et al., In: Interleukins, Lymphokines, and Cytokines (Oppenheim J.J., Cohen S., and Landy M., eds.) Academic Press New York 1983, pp. 609-615; Fisher P. et al., Proc. Natl. Acad. Sci. USA (1983) 80, 2961).

30

EXAMPLE 4

Anti-Interferon antibody treatment

The instant invention also encompasses a method of administering anti-interferon- α antibody(ies) that can be tolerated by the humans, produced either as monoclonal antibodies (as for instance mouse/human chimeric antibodies), as other chimeric combinations of antibodies, or as monoclonal antibodies produced by human hybridoma cells. Furthermore,
5 the invention encompasses the use of polyclonal antibodies produced in animals (e.g., pigs) or from transgenic animals (e.g., transgenic pigs) immunized with one or several species of human interferon- α . Antibodies to other cytokines that may be useful in the treatment of diabetes are also considered, among those, antibodies to tumor necrosis factor α , tumor necrosis factor β , etc.

10 It is possible to develop a strain of pigs which produces human immune globulins either together with the porcine immune globulins, which could be separated by affinity chromatography, or from a strain of pigs which produces human immune globulins exclusively. Previous work has achieved impressive clinical effect from HIV-1 hyperimmunized pigs that also have been immunized with a protein complex, named gp48
15 which is derived from cellular proteins (cell membrane associated) found in CD4+ cells such as H9 cell lines. The gp48 protein complex was first found to be present in HIV-1 lysate as described by Kurt Oster (Kurt B. Oster, U.S. Patent No. 5,286,852 incorporated by reference in the entirety). Later on the gp48 protein complex was identified in H9 cells and harvested from these cells, purified to be used as a separate immunogen composition in pigs
20 that were immunized with an antigen or antigens such as for instance tumor necrosis factor α in order to produce porcine immunoglobulins that at the same time would neutralize tumor necrosis factor α in humans and at the same time be tolerated well by the recipient due to the anti gp48 which among others contain antibodies against MHC class I and MHC class II. The anti gp48 antibodies will thus prevent the human from identifying the porcine
25 immunoglobulin as xenogenic or foreign.

Pigs immunized with human interferon- α specie(s) and with the gp48 protein complex, thereby rendering and thus produced porcine immune globulins capable of escaping the human immune surveillance system could then be useable in neutralizing circulating interferon- α specie(s) with tolerance identical to other porcine hyperimmune globulins such
30 as a porcine anti TNF- α immune globulins. It is anticipated that other species of animals may be escaping in the same manner as pigs provided these animals are co-immunized with gp48 protein complex or with antibodies to other T cell antigens such as gp39. The ligand for

CD40 which delivers signals to B cells can synergize with those provided by other B cell surface receptors to induce B cells proliferation and antibody class switching as well as modulating the cytokine production and cell adhesion. The ligand for CD40 has recently been found to be a cell surface protein of ~39kDa expressed by activated T cells, called gp39 (Hollenbaugh D. et al., The EMBO Journal (1992) 11, 4313). The gp39 is a type II membrane protein with homology to TNF.

The gp39 protein has been constructed and characterized in a soluble recombinant form. The results from Hollenbaugh et al.'s work has indicated that B cells require a second signal besides gp39-CD40 to drive proliferation and soluble gp39 alone in a non-membrane bound form is able to provide co-stimulatory signals to B cells. Noelle et al. has shown that monoclonal antibodies to the 39 kDa membrane protein or gp39 that is selectively expressed by T helper cells (CD4+ cells) inhibited CD40-Ig binding and also inhibited the activation of B cells by T helper cells (CD4+ cells) (Noelle R. et al., Proc. Natl. Acad. Sci., USA (1992) 89, 6550). Thus, it is a part of this invention to achieve porcine antibodies produced by co-immunizing pigs with gp39 together with the gp48 protein complex which, besides cloaking the MHC class I and class II may be capable of producing antibodies inhibiting the activation of B cells with regard to both antibody production and cytokine production, because blocking the binding gp39 with an antibody from binding with CD40 inhibits T-helper (CD4+) cell dependent B cell activation as shown by Noelle et al.

Porcine anti-interferon- α antibodies may in this way be constructed to be tolerated by humans with juvenile diabetes. It is possible that the mechanism with diabetes is also encompassing other cytokines such as tumor necrosis factor α in which case administration of anti TNF- α antibodies such as those that we produce in pigs, co-immunized with gp48 complex, and, possibly, also gp39 ligand.

25

EXAMPLE 5

Antisense technology

Blocking of interferon- α or interferon- α receptor synthesis using antisense technology

An antisense molecule capable of basepairing with DNA and/or RNA is introduced to the interior of the target cells. Inside the cells, the antisense molecule blocks synthesis

of the target protein by interfering with the DNA or RNA sequence needed for synthesis of the protein in question.

The antisense molecule

5 The antisense molecule can be a native nucleic acid (DNA or RNA) or a modified nucleic acid. As modified antisense molecules one can use a phosphorothioate, a methylphosphonate, a PNA (Peptide Nucleic Acid), or any of the modifications commonly practiced (see Crooke R.M. "In vitro toxicology and pharmacokinetics of antisense oligonucleotides," 1991, *Anti-Cancer Drug Design*, 6, 609-646.).

Introduction of antisense molecules to the target cells

10 The antisense molecule can be introduced to the interior of the target cells by unspecific methods, such as pinocytosis (Stein C.A. *et al.* "Dynamics of the Internalization of Phosphodiester Oligodeoxynucleotides in HL60 Cells," 1993, *Biochemistry*, 32, 4855-4861). The antisense molecules can also be introduced to the interior of the target cells by specific methods; the antisense molecule is conjugated to a molecule (a carrier molecule). The conjugate interacts specifically with receptors/molecules on the surface of the target cells (e.g., membrane receptors) and is thus presented to the interior of the cells by endocytose. As carrier molecules an antibody, a viral particle, or any molecule interacting with specific
15 receptors or molecules on the surface of the target cells (Walker I. *et al.* "Improved Cellular Delivery of Antisense Oligonucleotides Using Transferrin Receptor Antibody-Oligonucleotide Conjugates," 1995, *Pharmaceutical Research*, 12, 1548-1553) can be used.

Mechanism of blocking

25 The antisense can block synthesis of interferon α or interferon- α receptor on DNA level or RNA level (Crooke S.T. "Progress Toward Oligonucleotide Therapeutics: Pharmacodynamic Properties," 1993, *FASEB Journal*, 7, 533-539).

DNA level

The antisense can block transcription by

- 30 * Formation of triple-stranded structures
- * Binding to partially denatured DNA
- * Displacing one of the two strands in the DNA double helix

- * Cleavage of one or both strands by antisense-chelator conjugates

mRNA level

The antisense can block transcription by

- 5 * Binding to the translation initiation codon
- * Disrupting secondary RNA structure
- * Interfering with splicing
- * Enhancing degradation of mRNA by formation of RNA-DNA duplex, which is a specific substrate for Rnase H
- 10 * Interfering with capping at the 5-end or polyadenylation at the 3-end
- Cleaving the mRNA molecule

Interferon- α gene knockout in pancreatic β cells

Pancreatic cells are isolated and grown in vitro. These cells are then subjected to gene
15 knockout experiment to destroy their interferon- α production capability. This example describes the inactivation of the endogenous interferon- α gene by homologous recombination in these pancreatic cells. The strategy is to alter the interferon- α gene sequences with a gene targeting vector derived from this particular gene. This gene knockout vector is constructed to contain most of the genomic sequence of the interferon- α gene, a gene for neomycin
20 resistance (neor gene) and a disrupted piece of interferon- α exon.

Human interferon- α gene can be isolated from a genomic phage library derived from the isolated pancreatic β cells by PCR using portions of interferon- α nucleotide sequences as primers. The isolated interferon- α gene is then cloned and propagated in bacteria E. coli for example, to obtain pure interferon- α gene DNA in quantity for use in the construction of a
25 interferon- α gene knockout vector.

To construct the gene knockout vector, a *neo'* gene is first inserted into the coding sequence (exon) of the isolated interferon- α gene. The inserted neor gene will inactive the interferon- α gene by interrupting its correct gene sequence. It also services as a positive selection marker to promote the growth of cell that have incorporated the targeting vector. A
30 negative selection marker, the thymidine kinase (*tk*) gene from a herpes virus, is also added to one end of the inactivated gene. The *tk* gene will help eliminating most of the cells that have incorporated the targeting vector at a random location. Alternatively, the knock-out vector

can be engineered to carry a *neo^r* gene in noncoding sequences, flanking sequences or introns, and the truncated piece of exons (coding sequences). Once the knock-out vector is complete, it can be cloned and propagated in bacteria and introduced into the cultured pancreatic B cells by electroporation for homologous recombination to take place.

5 The injected vector will either take the place of the original gene through homologous recombination or fit itself randomly into a chromosome through random insertion or does not become integrated at all. The targeted insertion of vector DNA by homologous recombination can be selected by growing cells in selective media counting neomycin analogue (G418) and ganciclovir. G418 is lethal to cells unless they carry a functional *neo^r*
10 gene and so it eliminates cells in which no integration of vector has occurred. Meanwhile ganciclovir kills any cells that harbor a *tk* gene from a randomly integrated vector. Virtually the only cells actually have the recombination and knock out, Southern blot analysis with specific probe can be performed as a conformatory test.

 The cell line should contain a defective interferon- α gene and is ready for introduction
15 into patients.

EXAMPLE 6

Pancreatic Beta Cells

 An additional method of the instant invention would be to inactivate the interferon- α
20 gene by a gene knock-out technique described in EP-A-546073 (GenPharm International, Inc; incorporated by reference). A successful inhibition of interferon- α activity may also be accomplished by the effective alteration of the active residues involved in effecting binding.

 By the term "insulin producing cells" is herein meant either pancreatic β cells (preferably of human origin) or cells which have been genetically engineered so as to produce
25 insulin. One example of such genetically engineered cells could be a fibroblast (or any other suitable cell) which have been genetically manipulated by the introduction of the regulatory gene for human insulin, or in other words, introduction of the 5'-flanking region of the insulin gene containing two cis-acting elements, the enhancer and the promoter, which exert their effect on transcription by interacting with various trans-acting factors which normally restrict
30 insulin gene expression to the endocrine pancreas (Walker *et al.*, *Nature* 306, 557, 1983; Karlsson *et al.*, *Proc. Natl. Acad. Sci. USA* 84:8819, 1987, Moss *et al.*, *Mol. Cell. Biol.* 8:2620, 1988; and Ohlsson *et al.*, *Proc. Natl. Acad. Sci. USA* 85:4228, 1988).

By the term "an immunologically acceptable cell or cell line" is herein meant a cell or cell line which, upon introduction into the intended recipient, will not be rejected by the recipient. It is preferred that no medication will be necessary in order to accomplish this result (e.g. by using cells from the recipient himself) but it is contemplated that it will be necessary to use cells from donors which do not have a tissue type which is identical with that of the recipient. Therefore, a certain amount of immunosuppression may be required, but it should not include damaging chemotherapy on the recipient. It would be acceptable if the immunosuppression could be obtained by Cyclosporin, e.g. in combination with hydrocortisone or prednisolone as well as with azathioprine, a combination that has been used in allotransplantation of pancreas islet cell from a donor pig to a recipient pig that was rendered diabetic by a total pancreatectomy (Yamaguchi *et al.*, *Transplantation Proceedings* 24:1010, 1992). However, the method of using xenogenic islets in man would most probably give rise to rejection unless the human recipient receives heavy chemotherapy. An alternative way of performing successful pancreatic islet xenotransplantation may utilize the methods described by McCurry *et al.* by producing transgenic pigs that at the fertilization stage (ovum and/or spermatogonia) have received transfer of genes that regulate the human complement system at the level of the alternative complement component C3 pathway (McCurry *et al.*, *Nature Med.* 1:423, 1995) Another viable method could be to transfer the C1s esterase inhibitor gene in ova and/or spermatogonia in pigs and thereby producing transgenic pigs with a complement inhibition at the complement component C1/C4 level. However, it is anticipated that these types of xenogenic transplants of islets will most probably be rejected relatively fast in spite of the changes made by transgenic techniques.

By the term "transforming a cell" is herein meant the genetic manipulation of a cell which has as an effect that the cell changes its phenotype. Transformations of cells can be performed in a number of ways which are well-known in the art (cf. e.g. Maniatis *et al.*, Molecular Cloning: a laboratory manual, 2nd edition, Cold Spring Harbor Press, 1989). However, care should be taken that methods are not used which have as a result that interferon- α is expressed by the resulting transformed cell. For instance, transformation of cells capable of producing interferon- α by introduction of a viral carrier using CAT plasmids (Gorman *et al.*, *Mol. Cell. Biol.* 2:1044, 1982, and Edlund *et al.*, *Science* 230:912, 1985) or poly(dI-dC):poly(dI-dC) as used by Singh *et al.* (Singh *et al.*, *Nature* 319:154, 1986) should be avoided, as these are also potent interferon- α inducers. Thus, according to the instant

invention, the above-referenced methods (incorporated by reference) can be utilized to transform cells which will not or cannot be induced to produce interferon- α for the treatment of diabetes by insulin gene therapy.

Many methods for effective gene therapy are known in the art, for example United States Patent 5,399,346; 5,428,132; 4,868,116; 4,980,286; International Publication WO 89/02468; International Publication WO 92/07573; International Publication WO 92/07943; European Patent Application Publication 0 446 017 A1; and International Publication No. WO 94/12649 (all of which are hereby incorporated by reference in their entirety).

Therefore, when transferring to or generating transgenic cells in humans to induce an alternative self expressing cell type which is present *in vivo*, by isolating cells from a diabetic individual and transferring the regulatory region of the human insulin gene, followed by a re-introduction of the transgenic cells back to the diabetic patient (or to other diabetic patients of the same tissue type) as shown in the animal model by transferring the regulatory region of the human insulin gene or the porcine insulin gene, to a functionally same type of cells in the pig model followed by re-introduction into the pig that has been made diabetic (or to pigs of inbred or outbred types) will render the diabetic human or in the animal model, the diabetic pig able to utilize his or its transgenic cells to the production of *in vivo* insulin regulated by the regulatory region of the human insulin gene which constitutes this invention when the transgenic transfer is done to cells that do not at the same time co-express interferon- α .

If a cell type is chosen for the transgenic transfer of the regulatory region of the human insulin gene (or the porcine insulin gene), that later on differentiate into a interferon- α expressing cell, said cell type will be rendered incapable of contributing to the expression of and thereby production of insulin, if induced for interferon- α production. The cell may even develop an "insulinitis" like condition for instance by a chronic virus infection, or a condition that constantly induce interferon- α .

The ideal cell type to be used as an *in vivo* as insulin producer is one that will replicate and give rise to a cell population sufficiently large enough to produce part of or all the insulin needed *in vivo*, initiated by a regulatory region for human insulin gene (or porcine insulin gene) without being susceptible to being significantly depleted due to differentiation into co-expression of interferon- α . Such cells can be of human origin, or transgenic porcine cells, or other suitable mammalian cells. If the insulin producing transgenic cell population should decrease, an additional transgenic therapy could be repeated in the same type of cell

population or in other cell populations that fulfill the above criteria.

One could also transfer the regulatory region of the human (or porcine) insulin gene to a cell type that would replicate sufficiently to generate a significant insulin producing population, and where the cell type may not be able to differentiate into interferon- α production.

An additional method of the invention is to use anti-sense techniques to interrupt interferon- α expression in humans. Alternatively one could even treat diabetics with anti-interferon- α antibodies, such that one could diminish or even interrupt the further destruction of insulin producing β cells caused by the interferon- α . If this approach is used one may have to augment natural (in vivo) production of interferon- α by parenteral injection of exogenous interferon- α . This technique would be most effective in early stages of diabetes. There may be a possibility that the insulin producing β cells might regenerate under such a treatment approach.

A combination of transgenic transfer of the regulatory region of the human (or porcine) insulin gene to a non-expressing cell type to be re-introduced into a patient during an early onset of diabetes - together with the described antisense and/or with anti-interferon antibody therapy - may be an alternative treatment of diabetes that may approach a cure or at least an improvement of the early onset diabetes type I.

In patients with fully developed diabetes type I the transgenic treatment may be the preferred treatment in order to approach a cure or an improvement of the diabetic condition negating or diminishing the demand for exogenic regulation with insulin and at the same time keep the blood sugar reasonably under control, thereby approaching a better regulated diabetes type I, and complementing remaining endogenous insulin producing cells still functioning.

A patient with juvenile diabetes will still have circulating inducer(s) of interferon- α in cells capable of producing interferon- α and possibly other cytokines. It is not known whether β -interferon induction in cells necessarily interferes with a theoretical insulin production. Pancreatic β cells do not produce β -interferon. When "healthy" pancreatic islets are transplanted into patients with juvenile diabetes the patients will quickly destroy the insulin production in the cells transplanted. This would be expected if any interferon- α inducer(s) is "chronically" circulating in these patients.

The invention teaches that the ideal cell population for use to produce insulin does not

co-produce interferon- α or possibly even other cytokines and is thus functionally unresponsive, or incapable of producing functional cytokine. The ideal cell population can be selected from among fibroblast cells only producing β -interferon, or cell types that do not produce interferon at all. A well known cell line that does not produce interferon at all is the monkey kidney cell, Vero cell. This cell type has often been used in bioassays because no auto-induction of interferons occurs. This cell or cells lacking the above described function would be the ideal cell to introduce the regulatory gene for insulin. The concept would then be that the regulatory gene would activate the dormant insulin gene in the cell rendering it capable of producing insulin.

Thus the instant invention provides for a cell, capable of expressing insulin, but inhibited in the ability to produce interferon- α , for use in gene therapy and transplantation into diabetic patients. In one embodiment of the instant invention, the cell is capable of expressing insulin under the regulation of the human insulin inducer system, and incapable of expressing interferon- α , because of either knock-out mutation of the IF- α , or other genetic manipulation of IF- α expression. The preferred cell for such use is of human origin, but can be of transgenic origin whereby human Histocompatibility markers have been transferred and expressed by other animal cells. The cell of the instant invention can be capable of further differentiation, or preferably inhibited from further differentiation. The cell of the instant invention can actively divide, but for certain applications it may be better to have cells which are incapable of further cell division.

If the above described cell such as the Vero cell line was selected, - preferably a human cell that is not capable of producing at least interferon- α , as the transplanted cell after transfer of the genes necessary for insulin production and expression/secretion, it would not contain the gene(s) that are interfering with the insulin production as for instance cells that can not produce at least interferon- α . When the correct cell line(s) are selected and the genetic expression of insulin is introduced the cell line can then transplanted into the diabetic patient. Ideally, the order in sequence would be:

- Isolation of the cell from a juvenile diabetes patient
- Incorporation of the genes necessary for the cell to transgenically produce insulin
- Testing the cells selected for induction of interferon- α and, may be, other cytokines
- Re-implant the cell found not to be able to produce interferon- α (and other cytokines), now capable of producing insulin, into, ideally, the same patient from whom the cells

were harvested

- Monitor an eventual initiation/increase in insulin ~ smaller doses of insulin needed.

An animal model suitable for the insulin expressing experiments is the pig. The transfer of the regulatory region of the human or porcine insulin gene (or the entire genes needed to obtain porcine insulin gene expression) is being exemplified using a porcine model where the selected cells are isolated fibroblast cells, capable of producing β -interferon. and used as target cells for the regulatory region of the human insulin gene. A more ideal approach will be to isolate a porcine cell line that are not producing interferons or may be even not producing cytokines.

An alternative method would be to utilize a continuous porcine kidney cell line, PK15 for the construct of porcine or human regulatory region of the insulin gene or the entire genes involved in the insulin production. This cell line or a resembling cell line may be of advantage provided the cell lines selected do not produce interferon- α (or β -interferon). This model is advantageous because porcine insulin resembles that of humans to a degree where insulin from pigs have been used in human diabetics for several years. The pigs, treated so that they become diabetic, will be used in the some of the experiments. There are well-known ways of introducing diabetes in pigs. The difference between these pigs and human juvenile diabetes would be the fact that the pigs do not have a true diabetes.

Alternatively, it is possible to induce a chronic interferon production in pigs with intact pancreatic islet cell and insulin production in pigs with transferred transgenic cells that produce insulin. However, the main goal would be to utilize the transgenic techniques or the gene therapy for creating insulin producing cells to demonstrate the transfer the genes necessary to induce an insulin production in cells not used by the organism to produce insulin from said kind of cells. The insulin gene may be dormant in the cell being used for the gene transfer and thereby the gene transfer would be to operably introduce the regulatory region of the insulin gene in order to create the insulin production. What is yet to be learned is whether the insulin gene composition necessary for the cell to excrete insulin otherwise would be present, and/or whether other gene products necessary for an actual production and excretion of insulin from the cell would be possible.

The regulatory region of the porcine insulin gene is obtained using the procedure described previously (S.A. Chen *et al.*, *J. Interferon Res.* 8: 597, 1988; L. Martin *et al.*, *J.*

Immunol. 150: 1234, 1993; O. Bohoslawec *et al.*, *J. Interferon Res.* 6: 207, 1986).

The regulatory region of the porcine insulin gene is expressed in *E. coli*, *B. subtilis* or in other such as yeast or from cells containing said regulatory region.

Porcine kidney cells are selected and the regulatory region of the human insulin gene is transferred using the procedures described previously. The methods used have been described by Chen and Martin, who utilized the technology in transgenic mice by standard techniques without transferring the interferon- α transgene (L. Martin *et al.*, *J. Immunol.*, 150:1234, 1993). The pigs' pancreas is damaged by toxin so that the islets can not produce insulin, inducing a diabetes condition in the pig. The pig is treated with porcine insulin and the blood sugar is checked. The transgenic cells are re-introduced by infusion of the cells into kidney capsula and into peritoneum. The pig and the diabetic pig is controlled by weekly diminishing the insulin and controlling the blood sugar in order to see when the transgenic production of insulin starts and significantly diminish the need for administration of exogenous insulin.

EXAMPLE 7

Gene Therapy

In order to mimic the gene therapy in a diabetic patient, connective tissue is surgically, aseptically removed from the neck region and placed in Minimum Essential Medium (Earle's salt) with 10% fetal bovine serum. The cells are brought to the laboratory where the tissue is cut into cubes under a laminar air flow hood. The cubes are then cut in a petri dish into small cubicles. The cubicles are washed in MEM without fetal bovine serum and treated with 0.25% trypsin in MEM without fetal bovine serum. The trypsin treated cells are then transferred into a 25 ml tissue culture bottle. Five to 10 ml MEM with 5 to 10% fetal bovine serum is added to the tissue culture bottle. The cells are cultured in a 37°C incubator. The cells are checked daily. When a confluent cell culture has been obtained the cells are then trypsinized and transferred to a microinjection microscope. The regulatory region of the porcine or the human insulin gene is transferred using the process described under example 1. The cells into which the adequate genes are transferred are cultured in a 20 ml tissue culture bottle. When the cell count is sufficiently big (10^4 to 10^5 cells, or more) are then treated with 0.25% trypsin in MEM without fetal calf serum. When the cells are detached they are treated with MEM with 10% fetal bovine serum and will followingly be washed in sterile PBS at pH

7.2 to 7.4.

The pig that shall receive its own gene treated cells has in the meantime been rendered diabetic and exogenous insulin treatment has been initiated. The cells are injected into the peritoneum of the pig and intramuscularly into the neck region of the pig. The pig is
5 monitored for eventual production of endogenous insulin during a follow up time of at least 3 to 6 months. When the gene treated cells take over the insulin production the exogenous insulin needed by the pig should accordingly decrease.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Osther, Kurt B
- (ii) TITLE OF INVENTION: Method for Treating Diabetes
- (iii) NUMBER OF SEQUENCES: 2
- 10 (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: McDonnell Boehnen Hulbert & Berghoff, Ltd.
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- (C) CITY: Chicago
- 15 (D) STATE: Illinois
- (E) COUNTRY: USA
- (F) ZIP: 60606
- (v) COMPUTER READABLE FORM:
- 20 (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- 25 (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:
- 30 (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Chao, Mark
- (B) REGISTRATION NUMBER: 37,293
- (C) REFERENCE/DOCKET NUMBER: 95,192-E
- 35 (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: 312-913-0001
- (B) TELEFAX: 312-913-0002
- 40

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 189 amino acids

5 (B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

10

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..166

15 (D) OTHER INFORMATION: /note= "Swissprot P01562, human
interferon alpha Type I; possible A to V error at position 114."

(ix) FEATURE:

(A) NAME/KEY: Disulfide-bond

(B) LOCATION: 1..99

20

(ix) FEATURE:

(A) NAME/KEY: Disulfide-bond

(B) LOCATION: 29..139

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ala Ser Pro Phe Ala Leu Leu Met Val Leu Val Val Leu Ser Cys

-20

-15

-10

30

Lys Ser Ser Cys Ser Leu Gly Cys Asp Leu Pro Glu Thr His Ser Leu

-5

1

5

Asp Asn Arg Arg Thr Leu Met Leu Leu Ala Gln Met Ser Arg Ile Ser

10

15

20

25

35

Pro Ser Ser Cys Leu Met Asp Arg His Asp Phe Gly Phe Pro Gln Glu

30

35

40

Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Pro Ala Ile Ser Val Leu

40

45

50

55

```

His Glu Leu Ile Gln Gln Ile Phe Asn Leu Phe Thr Thr Lys Asp Ser
      60              65              70

5  Ser Ala Ala Trp Asp Glu Asp Leu Leu Asp Lys Phe Cys Thr Glu Leu
      75              80              85

Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Val Met Gln Glu Glu Arg
90              95              100              105

10 Val Gly Glu Thr Pro Leu Met Asn Ala Asp Ser Ile Leu Ala Val Lys
      110              115              120

Lys Tyr Phe Arg Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
15              125              130              135

Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Leu Ser
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20 Leu Ser Thr Asn Leu Gln Glu Arg Leu Arg Arg Lys Glu
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(2) INFORMATION FOR SEQ ID NO:2:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 195 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

30

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

35

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..172
- (D) OTHER INFORMATION: /note= "Swissprot P05000, Human
interferon alpha Type II"

40

(ix) FEATURE:

(A) NAME/KEY: Disulfide-bond

(B) LOCATION: 1..99

(ix) FEATURE:

5 (A) NAME/KEY: Disulfide-bond

(B) LOCATION: 29..139

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

10

Met Ala Leu Leu Phe Pro Leu Leu Ala Ala Leu Val Met Thr Ser Tyr
-20 -15 -10

15

Ser Pro Val Gly Ser Leu Gly Cys Asp Leu Pro Gln Asn His Gly Leu
-5 1 5

Leu Ser Arg Asn Thr Leu Val Leu Leu His Gln Met Arg Arg Ile Ser
10 15 20 25

20

Pro Phe Leu Cys Leu Lys Asp Arg Arg Asp Phe Arg Phe Pro Gln Asp
30 35 40

Met Val Lys Gly Ser Gln Leu Gln Lys Ala His Val Met Ser Val Leu
45 50 55

25

His Glu Asn Leu Gln Gln Ile Phe Ser Leu Phe His Thr Glu Arg Ser
60 65 70

30

Ser Ala Ala Trp Asn Asn Thr Leu Leu Asp Gln Leu His Thr Glu Leu
75 80 85

His Gln Gln Leu Gln His Leu Glu Thr Cys Leu Leu Gln Val Val Gly
90 95 100 105

35

Glu Gly Glu Ser Ala Gly Ala Ile Ser Ser Pro Ala Leu Thr Leu Arg
110 115 120

Arg Tyr Phe Gln Gly Ile Arg Val Tyr Leu Lys Glu Lys Lys Tyr Ser
125 130 135

40

Asp Cys Ala Trp Glu Val Val Arg Asn Glu Ile Asn Lys Ser Leu Phe
140 145 150

5 Leu Ser Thr Asn Asn Gln Glu Arg Leu Arg Ser Lys Asp Arg Asp Leu
155 160 165

Gly Ser Ser
170

10

What Is Claimed Is:

1. A method for treating early type I diabetes comprising administering an effective inhibitory amount of an interferon- α inhibitor.
2. A method as in Claim 1 wherein the inhibitor is selected from the group consisting of an antibody specific for IFN α , an anti-sense oligonucleotide homologous to at least a portion of the nucleic acid mRNA encoding for interferon- α , an anti-sense oligonucleotide homologous to at least a portion of the nucleic acid mRNA encoding for interferon- α receptor.
3. A method as in Claim 1 wherein the inhibitor administered is an effective therapeutic amount of anti-IFN α antibodies in combination with anti-sense oligonucleotide homologous to at least a portion of the nucleic acid mRNA sequence encoding IFN α .
4. A method for treating early type I diabetes comprising administering an effective inhibitory amount of a targeted inhibitor of an anti-viral cellular enzyme selected from the group consisting of 2',5' oligoadenylate synthetase (2',5' A synthetase), the La antigen (p47,6), and protein kinase p68 (PKR).
5. A method as in claim 6, where in the inhibitor is selected from the group consisting essentially of a double-stranded RNA which can inhibit the activity of an anti-viral cellular enzyme, a monoclonal antibody which can inhibit the activity of an anti-viral cellular enzyme, and a polyclonal antibody which can inhibit the activity of an anti-viral cellular enzyme.
6. A method for treatment of type I diabetes comprising administering cell constructs containing an inducible insulin promoter region operably linked to an expressible insulin gene, said cell being incapable of functional IFN α production, and resistant to IFN α activity.

7. A biological construct for the effective secretion of insulin comprising a cell which contains an inducible insulin promotor region operably linked to an expressible insulin gene, said cell being incapable of functional interferon- α production.
8. A method for treating type I diabetes comprising administering an effective number of insulin producing cells of Claim 7, to a mammal suffering from type I diabetes, said cells providing an effective therapeutic amount of insulin.
9. A medicament for the treatment of type I diabetes comprising a cell which contains an inducible insulin promotor region operably linked to an expressible insulin gene, said cell being incapable of functional interferon- α production.
10. A method for making a medicament for the treatment of type I diabetes comprising constructing a mammalian cell which contains an inducible insulin promotor region operably linked to an expressible insulin gene, said cell being incapable of functional interferon- α production.
11. A medicament for treating early type I diabetes comprising an effective inhibitory amount of an interferon- α inhibitor.
12. A method for making a medicament for treating early type I diabetes comprising combining an effective inhibitory amount of an interferon- α inhibitor with a suitable pharmaceutical carrier.
13. A medicament as in Claim 11 wherein the interferon- α inhibitor is an anti-sense oligonucleotide homologous to at least a portion of the nucleic acid mRNA encoding for interferon- α , an anti-sense oligonucleotide homologous to at least a portion of the nucleic acid mRNA encoding for interferon- α receptor, or an antibody or antigen binding fragment thereof which specifically binds to interferon- α or interferon- α receptor protein.

14. A method as in Claim 12 wherein the interferon- α inhibitor is an anti-sense oligonucleotide homologous to at least a portion of the nucleic acid mRNA encoding for interferon- α , an anti-sense oligonucleotide homologous to at least a portion of the nucleic acid mRNA encoding for interferon- α receptor, or an antibody or antigen binding fragment thereof which specifically binds to interferon- α or interferon- α receptor protein.
15. A medicament for treating early type I diabetes comprising an effective inhibitory amount of a targeted inhibitor of an anti-viral cellular enzyme selected from the group consisting of 2',5' oligoadenylate synthetase (2',5' A synthetase), the La antigen (p47,6), and protein kinase p68 (PKR).
16. A method for the preparation of a medicament for treating early type I diabetes comprising combining an effective inhibitory amount of a targeted inhibitor of an anti-viral cellular enzyme selected from the group consisting of 2',5' oligoadenylate synthetase (2',5' A synthetase), the La antigen (p47,6), and protein kinase p68 (PKR), with a suitable pharmaceutical carrier.
17. A medicament as in claim 15, wherein the inhibitor is a double-stranded RNA which can inhibit the activity of an anti-viral cellular enzyme, a monoclonal or a polyclonal antibody which can inhibit the activity of an anti-viral cellular enzyme.
18. A method as in claim 16, wherein the inhibitor is a double-stranded RNA which can inhibit the activity of an anti-viral cellular enzyme, a monoclonal or a polyclonal antibody which can inhibit the activity of an anti-viral cellular enzyme.

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